A---

PATENT
139096-00030

4/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Kenten, J. et al.
Serial No.	:	09/480,544
Filed	:	January 10, 2000
For	:	CYCLING DNA/RNA AMPLIFICATION ELECTROCHEMILUINESCENT PROBE ASSAY
Group Art Unit	:	1655
Examiner	:	A. Chakrabarti

919 Third Avenue
New York, New York 10022

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on October 17, 2000

Barry Evans, Reg. No. 22,802
Name of Applicant, Assignee or Registered Representative

Signature

10/17/00
Date of Signature

AMENDMENT

Sir:

In response to the Official Action mailed April 17, 2000, Applicants respectfully request entry of the following amendment.

IN THE SPECIFICATION:

As and for a first paragraph of the specification on page 1, following the title, insert the following text: -- This application is a continuation of application Serial No. 08/474,927, filed June 7, 1995, now U.S. Patent No. 6,048,687, which was a continuation of application Serial No. 08/124,686, filed September 22, 1993, now abandoned. --

IN THE CLAIMS:

Cancel claims 1-20, all of the claims in this application.

Enter the following new claims:

21. A process for the detection of a specific nucleic acid sequence, comprising:

(a) obtaining a sample suspected of containing the specific nucleic acid sequence:

(b) adding the sample to a reagent mixture to form a reaction mixture wherein the reagent mixture comprises,

(i) a first oligonucleotide primer;

(ii) a second oligonucleotide primer comprising an antisense sequence of a promoter;

(iii) a DNA-directed RNA polymerase that recognizes said promoter;

(iv) an RNA-directed DNA polymerase;

(v) a DNA-directed DNA polymerase;

(vi) a ribonuclease that hydrolyses RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA:

(c) incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence to form an amplified nucleic acid sequence mixture;

(d) obtaining samples from said amplified nucleic acid sequence mixture;

(e) adding to said sample from the amplified nucleic acid sequence mixture of the following reagents:

(i) at least one probe sequence which specifically hybridizes to said RNA first template labeled with an electrochemiluminescent species;

(ii) at least one second capture probe sequence which specifically hybridizes to said RNA first template labeled with a binding species;

(iii) a bead coated with a binding species to said second probe sequence, to form a second mixture;

(f) incubating the second mixture for a time sufficient to allow hybridization between said probe(s) and first template molecule to form a complex; and

(g) detecting said bead bound complex using said electrochemiluminescent species.

22. The process of claim 21 wherein biotinylated or ECL labelled nucleotides are added to step (b) in sufficient quantity to produce, respectively, biotinylated or ECL labelled amplified target molecules.

23. The process of claim 21 wherein a biotinylated or ECL labelled primer is substituted for either primer 1 or primer 2 of step (b) to form a biotinylated or ECL labelled amplified target molecule.

24. The process of claim 21 wherein the labelled binding moiety specific for the nucleic acid hybridization complex formed in step (f) is added to step (e).

25. The process of claim 21 wherein the strepavidin is covalently linked to the bead.

26. The process of claim 21 wherein strepavidin is coated on the beads.

27. The process of claim 21 wherein the biotin labelled oligonucleotide probe is pre-incubated with the strepavidin bead to form a complex of the bead and the probe linked via a strepavidin-biotin interaction.

28. The process of claim 21 wherein the labelled capture probe and labelled detector probe are specific to distinct portions of the sequence of the amplified target molecule.

29. The process of claim 21 wherein the incubating conditions are those which permit:

(i) said first oligonucleotide primer to hybridize to said RNA first template;

(ii) said RNA-directed DNA polymerase to hybridize to utilize said RNA first template to synthesize a DNA second template by extension of said first oligonucleotide primer and thereby form an RNA-DNA hybrid intermediate;

(iii) said ribonuclease to hydrolyse RNA contained in said RNA-DNA hybrid intermediate;

(iv) said second oligonucleotide primer to hybridize to said DNA second template;

(v) said DNA-directed DNA polymerase to utilize said second oligonucleotide primer as template to synthesize said promoter by extension of said DNA second template; and

(vi) said DNA-directed RNA polymerase to recognize said promoter and transcribes said DNA second template.

30. In a cycling DNA/RNA amplification assay involving an initial nucleic acid template and having at least one amplification cycle which results in an amplification reaction mixture wherein the improvement comprises:

(a) obtaining samples from the amplification reaction mixture;

(b) adding to said sample the following reagent mixture composed of,

(i) at least one probe sequence which specifically hybridizes to said initial nucleic acid template and labelled with an electrochemiluminescent species;

(ii) at least one second capture probe sequence which specifically hybridizes to said initial nucleic acid template and labelled with a binding species;

(iii) a bead coated with a binding species to said second probe sequence;

(c) proving conditions of temperature and buffer to allow hybridization of the probes to said first nucleic acid template and the binding of said binding species on said second capture probe with the binding species or said bead to form a bead bound complex;

(d) detecting said bead bound complex using said electrochemiluminescent species.

31. A process for the detection of a specific nucleic acid sequence, comprising:

(a) obtaining a sample suspected of containing the specific nucleic acid sequence:

(b) amplifying said nucleic acid sequence in the presence of suitable oligonucleotide primers, polymerases and a ribonuclease that hydrolyses the RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, to form an amplified nucleic acid sequence mixture containing an RNA sequence complementary to said amplified DNA sequence;

(c) obtaining a sample from said amplified nucleic acid sequence mixture;

(d) adding to said sample from said amplified nucleic acid sequence mixture the following reagents:

(i) a first probe sequence which specifically hybridizes to said RNA first sequence, said probe being labeled with an electrochemiluminescent specie,

(ii) at least one capture probe sequence which specifically hybridizes to said RNA sequence, said capture probe being labeled with a binding species, and

(iii) a bead coated with a binding species to said second probe sequence, and thereby forming a second mixture;

(e) incubating the second mixture for a time sufficient to allow hybridization between said probe(s) and said RNA sequence to form a complex; and

(f) detecting said bead bound complex using said electrochemiluminescent species.

REMARKS

The specification has been amended to include a recitation of the applications in the family tree of the captioned application. More specifically, the specification now reflects that this application is a continuation of application Serial No. 08/474,927 filed June 7, 1995, now U.S. Patent No. 6,048,687, which application was itself a continuation of application Serial No. 08/124,686, filed September 22, 1993, now abandoned.

All of the claims have been cancelled and replaced with new claims 21-31.

The rejections of the claims under 35 U.S.C. §102 and 35 U.S.C. §103 for the reasons set forth in paragraphs 5, 6, 7 and 8 of the Official Action are respectfully traversed for the single reason that all of those rejections rely on Earle et al, U.S. Patent No. 5,925,518, filed May 23, 1995. It is respectfully pointed out that the subject application is ultimately a continuation of application Serial No. 08/124,686, filed September 22, 1993 and that date substantially precedes the filing date of the Earle et al. reference. Accordingly, all of the rejections set forth are respectfully believed to be unfounded.

Favorable reconsideration of this application is respectfully requested.

Respectfully submitted,

KRAMER LEVIN NAFTALIS & FRANKEL LLP
Attorneys for Applicants

By: _____

Barry Evans
Reg. No. 22,802
(212) 715-7609